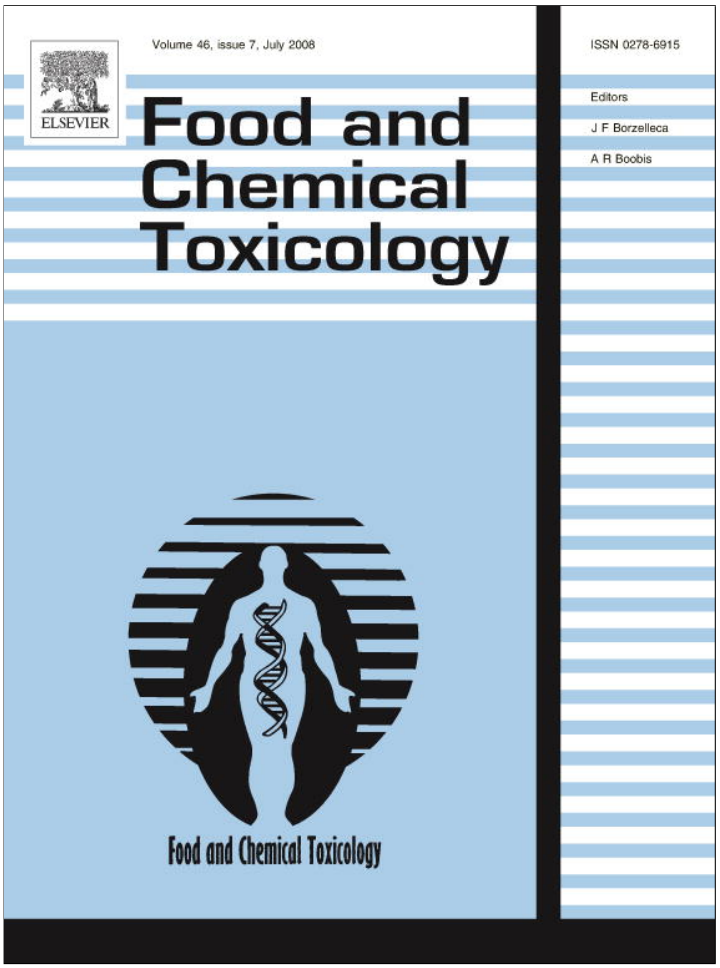

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Total phenols, antioxidant potential and antimicrobial activity of walnut (*Juglans regia* L.) green husks

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ABSTRACT

The total phenols content and antioxidant and antimicrobial activities were studied in walnut (*Juglans regia* L.) green husks aqueous extracts of five different cultivars (Franquette, Mayette, Marbot, Mellanaise and Parisienne). Total phenols content was determined by colorimetric assay and their amount ranged from 32.61 mg/g of GAE (*cv*. Mellanaise) to 74.08 mg/g of GAE t (*cv*. Franquette). The antioxidant capacity of aqueous extracts was assessed through reducing power assay, scavenging effects on DPPH (2,2-diphenyl-1-picrylhydrazyl) radicals and β-carotene linoleate model system. A concentration-dependent antioxidative capacity was verified in reducing power and DPPH assays, with EC_{50} values lower than 1 mg/mL for all the tested extracts. The antimicrobial capacity was screened against Gram positive and Gram negative bacteria, and fungi. All the extracts inhibited the growth of Gram positive bacteria, being *Staphylococcus aureus* the most susceptible one with MIC of 0.1 mg/mL for all the extracts. The results obtained indicate that walnut green husks may become important in the obtainment of a noticeable source of compounds with health protective potential and antimicrobial activity.

1. Introduction

Walnut (*Juglans regia* L.) is a valuable crop being the nut very popular and largely consumed. In Portugal, this species is widely spread throughout the country. Not only dry fruit (nuts) are used but also green walnuts, shells, kernels, bark, green walnut husks (epicarp) and leaves have been used in both cosmetic and pharmaceutical industry (Stampar et al., 2006).

In recent decades an increasing tendency towards the use of natural substances instead of the synthetic ones has been observed. As the synthetic materials and products are more complex in comparison to natural substances, it will take a long time for them to complete their natural cycles and return to nature; thus causing a lot of environmental pollution. Also with the increase in the price of raw materials, the problem of cost benefits for chemical production is becoming more considerable. Natural antioxidants, such as phenolic compounds, used as natural antioxidants, are gaining importance, due to their benefits for human health, decreasing the risk of degenerative diseases by reduction of oxidative stress and inhibition of macromolecular oxidation (Silva et al., 2004; Pulido et al., 2000; Tseng et al., 1997). Their use as preserving food additives (Zupko et al., 2001) had increasing interest.

In addition to antioxidant activity, several studies demonstrated the antimicrobial activity of phenols and/or phenolic extracts (Fernández et al., 1996; Hoult and Payá, 1996; Pereira et al., 2006, 2007a,b; Proestos et al., 2005; Puupponen-Pimiä et al., 2001; Rauha et al., 2000; Sousa et al., 2006; Zhu et al., 2004), making them a good alternative to antibiotics and chemical preservatives. There is an extended interest in using natural antimicrobial compounds, as the consumer's pressure on the food industry augments, to avoid chemical preservatives and due to the increasing resistance to antibiotics (Oliveira et al., 2007; Cowan, 1999).

Walnut's green husk is a by-product of the walnut production, having scarce use. Thus, using husk as a source of phytochemicals will increase the value of the walnut production, as well as offer utilization for a by-product, which is produced in a large quantity.

Different works demonstrated the potential antioxidant of walnut products, especially fruits (Espín et al., 2000; Li et al., 2006, 2007; Pereira et al., 2008) but also leaves (Pereira et al., 2007b) and liqueurs produced by green fruits (Stampar et al., 2006). Studies have also demonstrated the antimicrobial activity of walnut products, particularly of bark (Alkhawajah, 1997), leaves (Clark et al., 1990; Pereira et al., 2007b), fruits (Pereira et al., 2008) and the specific compound juglone (Clark et al., 1990) but until this time the information about walnut green husks is almost inexistent.

In the present work, green husk (Fig. 1) from five walnut cultivars (*cv*. Franquette, Marbot, Mayette, Mellanaise and Parisienne) grown in Portugal, were studied, regarding their total phenols content, antioxidant and antimicrobial activities. Antioxidant potential

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Fig. 1. Walnut green husks.

was accessed by the reducing power assay, the scavenging effect on DPPH (2,2-diphenyl-1-picrylhydrazyl) radicals and β-carotene linoleate model system. Antimicrobial capacity was also accessed, against Gram positive (*Bacillus cereus*, *B. subtilis, Staphylococcus aureus*) and Gram negative bacteria (*Pseudomonas aeruginosa*, *Escherichia coli, Klebsiella pneumoniae*) and fungi (*Candida albicans*, *Cryptococcus neoformans*).

2. Materials and methods

2.1. Samples

Walnuts' green husks were obtained from five *J. regia* L. cultivars: Franquette, Marbot, Mayette, Mellanaise and Parisienne that were collected on October 2006 in Bragança, northeast of Portugal (41° 47' 47.50918" N, 6° 46' 5.71990" W, 744.341 m). The orchard has a planting density of 3.5 × 7 m. The trees are ten years old, being pruned when necessary. No phytosanitary treatments were applied. Per cultivar, approximately 2 kg of fruits were handpicked from the soil, and the walnut green husks were removed, put in plastic bags and immediately frozen at −20°. The plant material was then freeze dried.

2.2. Samples preparation

Before each kind of analysis (total phenols determination, antioxidant and antimicrobial activities assays) the walnuts' green husk were extracted with 250 mL of boiling water for 45 min, and filtered through Whatman no. 4 paper (for each cultivar, three powdered subsamples, 5 g, 20 mesh). The aqueous extracts were frozen, lyophilized and redissolved in water at concentrations of 100 mg/mL and 10 mg/mL for antimicrobial and antioxidant activities assays, respectively.

2.3. Determination of total phenols content

Total phenols content in the obtained extracts were estimated by a colorimetric assay based on procedures described by Singleton and Rossi (1965) with some modifications. Briefly, 1 mL of sample was mixed with 1 mL of Folin and Ciocalteu's phenol reagent. After 3 min, 1 mL of saturated sodium carbonate solution was added to the mixture and adjusted to 10 mL with distilled water. The reaction was kept in the dark for 90 min, after which the absorbance was read at 725 nm (Analytik Jena 200–2004 spectrophotometer). Gallic acid was used for constructing the standard curve (0.01–0.4 mM). The results are expressed as mg of gallic acid equivalents/g of extract (GAEs).

2.4. Antioxidant Activity

2.4.1. Reagents

BHA (2-*tert*-butyl-4-methoxyphenol), TBHQ (*tert*-butylhydroquinone) and α-tocopherol were purchased from Sigma (St. Louis, MO, USA). 2,2-Diphenyl-1-picrylhydrazyl (DPPH) was obtained from Alfa Aesar. All other chemicals were obtained from Sigma Chemical Co. (St. Louis, USA). Methanol was obtained from Pronalab (Lisboa, Portugal). Water was treated in a Mili-Q water purification system (TGI Pure Water Systems, USA).

2.4.2. Reducing power assay

The reducing power was determined according to a described procedure (Oyaizu, 1986; Ferreira et al., 2007). Various concentrations of sample extracts (2.5 mL) were mixed with 2.5 mL of 200 mmol/L sodium phosphate buffer (pH 6.6) and 2.5 mL of 1% potassium ferricyanide. The mixture was incubated at 50 °C for 20 min. After incubation 2.5 mL of 10% tricloroacetic acid (w/v) were added and then the mixture was centrifuged at 1000 rpm in a refrigerated centrifuge (Centorion K24OR- 2003), for 8 min. The upper layer (5 mL) was mixed with 5 mL of deionised water and 1 mL of 0.1% of ferric chloride, and the absorbance was measured spectrophotometrically at 700 nm. The extract concentration providing 0.5 of absorbance (EC_{50}) was calculated from the graph of absorbance registered at 700 nm against the correspondent extract concentration. BHA and α-tocopherol were used as reference compounds.

2.4.3. Scavenging effect assay

The capacity to scavenge the 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical was monitored according to a method reported before (Hatano et al., 1988). Various concentrations of sample extracts (0.3 mL) were mixed with 2.7 mL of methanolic solution containing DPPH radicals (6×10^{-5} mol/L). The mixture was shaken vigorously and left to stand in the dark until stable absorption values were obtained. The reduction of the DPPH radical was measured by monitoring continuously the decrease of absorption at 517 nm. DPPH scavenging effect was calculated as percentage of DPPH discolouration using the equation: % scavenging effect = $[(A_{DPPH} - A_S)/A_{DPPH}] \times 100$, where A_S is the absorbance of the solution when the sample extract has been added at a particular level and A_{DPPH} is the absorbance of the DPPH solution. The extract concentration providing 25% inhibition (EC_{25}) was calculated from the graph of scavenging effect percentage against extract concentration. BHA and α-tocopherol were used as reference compounds.

2.4.4. β-Carotene linoleate model system

The antioxidant activity of walnut leaf extracts was evaluated according to a described procedure (Mi-Yae et al., 2003) β-carotene solution was prepared by dissolving 2 mg of β-carotene in 10 mL of chloroform. Two millilitres of this solution were placed in a 100 mL round-bottom flask. After chloroform removal, at 40 °C under vacuum, 40 mg of linoleic acid, 400 mg of Tween 80 emulsifier, and 100 mL of distilled water were added to the flask under vigorous shaking. Aliquots (4.8 mL) of this emulsion were transferred into different test tubes containing 0.2 mL of different concentrations of walnut leaf extracts. The tubes were shaken and incubated at 50 °C in a water bath. As soon as the emulsion was added to each tube, the zero time absorbance at 470 nm was measured. Absorbance readings were then recorded until the control sample had changed colour. A blank assay, devoid of β-carotene, was prepared for background subtraction. Antioxidant activity was calculated using the following equation: Antioxidant activity = (β-carotene content after 2 h of assay/initial β-carotene content) × 100. The assays were carried out in triplicate and the results were expressed as mean values ± standard deviations. The extract concentration providing 50% antioxidant activity (EC_{50}) was calculated from the graph of antioxidant percentage against extract concentration. TBHQ was used as reference compound.

2.5. Antimicrobial activity

2.5.1. Reagents

Ampicillin and cycloheximide were of the highest available quality, and purchased from Merck (Darmstadt, Germany). Water was treated in a Mili-Q water purification system (TGI Pure Water Systems, USA).

2.5.2. Microorganisms and culture conditions

Microorganisms CECT were obtained from the Spanish type culture collection (CECT) of Valencia University, while microorganisms ESA were clinically isolated strains identified in Microbiology Laboratory of Escola Superior Agrária de Bragança. Gram + (*Bacillus cereus* CECT 148, *B. subtilis* CECT 498 and *Staphylococus aureus* ESA 7 isolated from pus) and Gram – (*Escherichia coli* CECT 101, *Pseudomonas aeruginosa* CECT 108 and *Klebsiella pneumoniae* ESA 8 isolated from urine) bacteria, and fungi (*Candida albicans* CECT 1394 and *Cryptococcus neoformans* ESA 3 isolated from vaginal fluid) were used to screen antimicrobial activity of the three walnut cultivars. Microorganisms were cultured aerobically at 37 °C (Scientific 222 oven model, 2003) in nutrient agar medium for bacteria, and at 30 °C (Scientific 222 oven model, 2003) in sabouraud dextrose agar medium for fungi.

2.5.3. Test assays for antimicrobial activity

The screening of antibacterial activities against Gram + and Gram - bacteria and fungi and the determination of the minimal inhibitory concentration (MIC) were achieved by an adaptation of the agar streak dilution method based on radial diffu-

sion (Ferreira et al., 2004; Sousa et al., 2006). Suspensions of the microorganism were prepared to contain approximately 10^8 cfu/mL, and the plates containing agar medium were inoculated (100 μL; spread on the surface). Each sample (50 μL) was placed in a hole (3 mm depth, 4 mm diameter) made in the centre of the agar. Under the same conditions, different solutions of ampicillin (antibacterial) and cycloheximide (antifungal) were used as standards. The MIC was considered to be the lowest concentration of the tested sample able to inhibit the growth of bacteria after 24 h and fungi after 48 h. The diameters of the inhibition zones corresponding to the MICs were measured using a ruler, with an accuracy of 0.5 mm. Each inhibition zone diameter was measured three times (three different plates) and the average was considered. A control using only inoculation was also carried out.

3. Results and discussion

3.1. Total phenols content

The total phenols content in walnut green husks aqueous extracts was different according to the variety. *cv*. Franquette showed the high amount of these compounds, with 74.08 mg/g of GAE, being 1.7–2.3-fold higher than for other varieties. The lowest amounts were obtained for Mellanaise aqueous extracts with 32.61 mg/g of GAE (Table 1). The extraction yields were very similar in the different walnut varieties ranging from 31.63 and 33.69%.

A few studies were developed concerning the phenolic composition of green walnut husks. Juglone (5-hydroxy-1,4-naphthoquinone) is known as a characteristic compound of *Juglans* spp. being reported its occurrence in green walnut husks (Mahoney et al., 2000). These authors report also the existence of other naphtoquinones. On the other hand, Stampar et al. (2006) identified thirteen phenolic compounds in walnut husks: chlorogenic acid, caffeic acid, ferulic acid, sinapic acid, gallic acid, ellagic acid, protocatechuic acid, syringic acid, vanillic acid, catechin, epicatechin, myricetin, and juglone. However, in the study developed by Stampar et al. (2006) they collected walnuts with green husk just before the hardening of the endocarp, and probably this fact could interfere in the identified compounds. Recently, Pereira et al. (2007b) reported, in walnut leaves, the identification and quantification of ten phenolic compounds, namely 3- and 5-caffeoylquinic acids, 3- and 4-*p*-coumaroylquinic acids, *p*-coumaric acid, quercetin 3-galactoside, quercetin 3-pentoside derivative, quercetin 3-arabinoside, quercetin 3-xyloside and quercetin 3-rhamnoside. In those matrices the main constituent was found to be quercetin 3-galactoside (Amaral et al., 2004; Pereira et al., 2007b).

3.2. Antioxidant activity

In this study, the antioxidant capacity against ROS species of walnut green husk samples was accessed by three different assays: reducing power, scavenging activity on DPPH radicals and lipid peroxidation inhibition by β-carotene-linoleate system. ROS occurs in foods and are mainly responsible for the initiation of oxidation reaction. ROS react with lipids, proteins, sugars and vitamins, producing undesirable volatile compounds, destroying essential fatty acids, amino acids and vitamins and producing carcinogens (Almeida et al., 2008), and make food products less acceptable or unacceptable to consumers. In biological systems, oxidative stress, resulting from an imbalance between the generation of ROS and the antioxidant defence capacity of the cell affects major cellular components, including lipids, proteins and DNA. Continuous overproduction of ROS and/or the decrease in antioxidant defences may contribute to the development of several hearth diseases (Valko et al., 2007).

Green walnut husks extracts revealed a strong reducing power. The reducing power of a compound may serve as a significant indicator of its potential antioxidant activity (Meir et al., 1995). The presence of reducers (i.e. antioxidants) causes the reduction of the Fe^{3+}/ferricyanide complex to the ferrous form (Fe^{2+}) monitored at 700 nm (Sousa et al., 2008). In this assay, the yellow colour of the test solution changes to green depending on the reducing power of the test specimen. All the aqueous walnut husks extracts presented a concentration-dependent activity (Fig. 2) increasing the absorbance at 700 nm with increasing concentration. At a very low extract concentrations (0.1 mg/mL) the absorbance at 700 nm varied from 0.09 (*cv*. Mellanaise) and 0.18 (*cv*. Franquette). To obtain similar absorbance values with the tested standards we need to increase 36-fold for BHA (abs at 700 = 0.12 at 3.6 mg/mL) and 86-fold for α-tocopherol (abs at 700 = 0.13 at 8.6 mg/mL). For extract concentrations of 1 mg/mL the reducing power values varied from 1.01 (*cv*. Mellanaise) to 2.00 (*cv*. Franquette) (Fig. 2). EC_{50} values obtained for aqueous walnut husks extracts were lower than 1.8 mg/mL. In general, extracts with high total phenols content presented lower EC_{50} values in reducing power assay and in the order Franquette < Marbot < Parisinne < Mayette < Mellanaise (Table 2).

DPPH assay has been widely used to determine the free radical-scavenging activity of various plants and pure compounds (Pereira et al., 2006; Ferreira et al., 2007; Sousa et al., 2008). DPPH is a stable free radical which dissolves in methanol, and its purple colour shows a characteristic absorption at 517 nm. Antioxidant molecules scavenge the free radical by hydrogen donation, the colour from the DPPH assay solution becomes light yellow resulting in a decrease in absorbance at 517 nm. Free radical scavenging is one of the known mechanisms by which antioxidants inhibit lipid oxidation (Ferreres et al., 2007). In this assay, results are expressed as the ratio percentage of the absorbance decrease of DPPH radical solution in the presence of extract at 517 nm to the absorbance of DPPH radical solution at the same wave length. In the present work, the scavenging effect on DPPH radicals assay also showed concentration-dependent activity (Fig. 3). For example to the aqueous extract of walnut husks *cv*. Franquette, at 0.01 mg/mL, presented a scavenging effect of 3.81% that increase to 91.81% at 1 mg/mL. All the studied extracts exhibited high scavenging properties against DPPH radicals, varying, at 1 mg/mL extract concentration, from 83.16% (*cv*. Mellanaise) and 93.92% (*cv*. Marbot). The obtained results showed a strong antioxidant potential when comparing to the ones obtained for the standards (BHA: 96% at 3.6 mg/mL and α-tocopherol: 95% at 8.6 mg/mL). On DPPH assay, EC_{50} values obtained for aqueous walnut husks extracts varied between 0.35 mg/mL and 0.59 mg/mL in order of Franquette < Marbot < Parisinne = Mayette < Mellanaise (Table 2). Samples with higher total phenols showed the strongest free radical-scavenging effect (lower EC_{50} values), being established a significantly negative linear correlation between the total phenols content and EC_{50} values (determination coefficients 0.795; $p = 0.042$).

In β-carotene linoleate model system free radical arisen from oxidation of linoleic acid, attacks the highly unsaturated β-carotene molecules, causing decrease of the absorbance at 470 nm. The presence of different antioxidants can hinder the extent of β-carotene bleaching by neutralizing the linoleate-free radical and other free radicals formed in the system (Jayaprakasha et al., 2001). In the absence of antioxidants the absorbance at 470 nm

Table 1
Extraction yield (in percentage) and total phenols content (mg GAEs/g) aqueous extracts of walnut green husks from the Franquette, Mayette, Marbot, Mellanaise and Parisienne cultivars

Cultivar	Extraction yield (%)	Total phenols contents
Franquette	31.63 ± 0.98	74.08 ± 0.02
Marbot	33.30 ± 3.33	43.77 ± 0.01
Mayette	32.19 ± 1.46	41.45 ± 0.01
Mellanaise	32.41 ± 3.75	32.61 ± 0.01
Parisienne	33.69 ± 1.24	38.76 ± 0.01

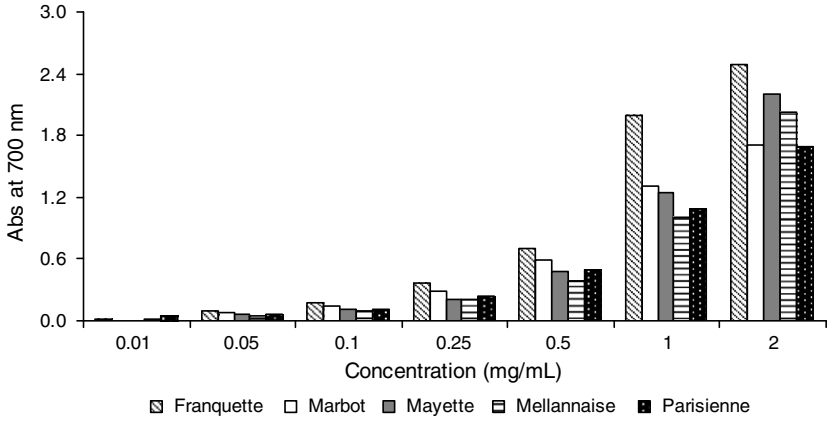

Fig. 2. Aqueous extracts reducing power values of walnut green husks from the Franquette, Mayette, Marbot, Mellanaise and Parisienne cultivars. Each value is expressed as mean ± standard deviation.

Table 2
EC_{50} values (mg/mL) aqueous extracts of walnut green husks from the Franquette, Mayette, Marbot, Mellanaise and Parisienne cultivars

Cultivar	Reducing power (EC_{50})	DPPH (EC_{50})	β-Carotene bleaching (EC_{25})
Franquette	0.50	0.35	1.27
Marbot	0.51	0.42	0.60
Mayette	0.67	0.51	0.10
Mellanaise	0.70	0.59	1.17
Parisienne	0.62	0.51	0.92

decreases rapidly, whereas in their presence, the colour, and thus absorbance, is retained for a longer time. The antioxidant activity of walnut green husks extracts, measured by the inhibition of β-carotene bleaching is shown in Fig. 4. The results showed a concentration-dependent antioxidant capacity and EC_{25} values varied from 0.1 mg/mL (*cv.* Mayette) and 1.25 mg/mL (*cv.* Franquette) (Table 2).

cv. Franquette proved to possess a much higher content of total phenols than all of the other cultivars. This occurrence can be related to the results obtained for the antioxidant assays, as *cv.* Franquette presents the higher values of total phenols and the better results for reducing power and scavenging activity on DPPH radicals assays, while *cv.* Mellanaise presents the worst results and the lower content of total phenols. In fact, several studies pointed out the antioxidant potential of phenols (Pereira et al., 2006; Ferreira et al., 2007; Ramadan and Moersel, 2006; Kornsteiner et al., 2006).

3.3. Antimicrobial activity

The study of antimicrobial capacity of plant phenolics is well-known (Pereira et al., 2006; Pereira et al., 2007a,b; Proestos et al., 2005; Rauha et al., 2000). However, crude extracts tested by our group with other natural products (fruits and leaves) also showed good antimicrobial activities, such as tables olives (Pereira et al., 2006; Sousa et al., 2006), hazelnuts (Oliveira et al., 2007), walnuts (Pereira et al., in press), walnut leaves (Pereira et al., 2007b), olive leaves (Pereira et al., 2007a), hazelnut leaves (Oliveira et al., 2007) and different Brassica species flower buds (Sousa et al., in press). In this work, we propose the use of walnut green husks, a by-product of walnut production, as antimicrobials source. Borchers et al. (2004) reported that crude extracts may be more beneficial than isolated constituents, since a bioactive individual component can change its properties in the presence of other compounds present in the extracts.

The walnut green husks aqueous of different cultivars were screened for their antimicrobial properties against *B. cereus*, *B. subtilis*, *S. aureus*, *E. coli*, *P. aeruginosa*, *K. pneumoniae*, *C. albicans* and *C. neoformans*. The minimal inhibitory concentration (MIC) values for the tested bacteria and fungi were determined as an evaluation of the antimicrobial activity of the tested extracts. The halos of the inhibition zones corresponding to the MICs are also presented (Table 3). The response for each microorganism tested was different. All the tested extracts revealed antimicrobial activity showing different selectivity and MICs for each microorganism.

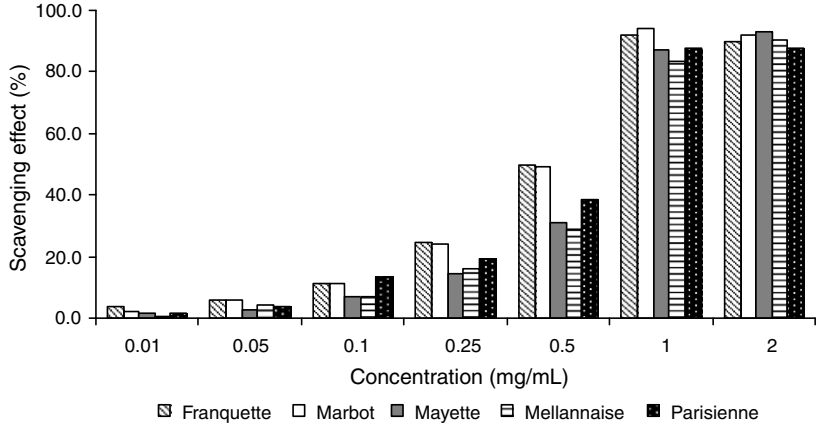

Fig. 3. Aqueous extracts scavenging effect on DPPH of walnut green husks from the Franquette, Mayette, Marbot, Mellanaise and Parisienne cultivars. Each value is expressed as mean ± standard deviation.

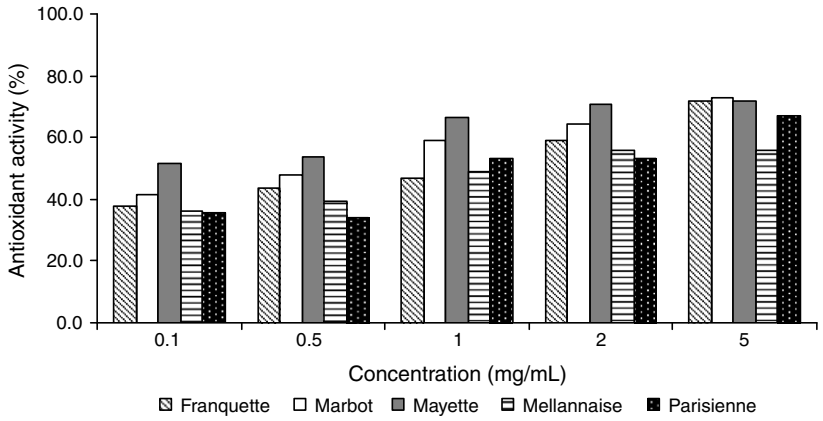

Fig. 4. Aqueous extracts antioxidant activity (%) by β-carotene bleaching method of walnut green husks from the Franquette, Mayette, Marbot, Mellanaise and Parisienne cultivars. Each value is expressed as mean ± standard deviation.

Table 3
Antimicrobial activity of aqueous extracts of walnut green husks from different cultivars

Cultivar	MIC (mg/mL)							
	B. cereus	*B. subtilis*	*S. aureus*	*P. aeruginosa*	*E. coli*	*K. pneumoniae*	*C. albicans*	*C. neoformans*
Franquette	0.1 (+ + + +)	10 (+ + +)	0.1 (+ + + +)	100 (+ +)	100 (−)	100 (−)	100 (−)	100 (−)
Marbot	1 (+ + + +)	0.1 (+ + +)	0.1 (+ + + +)	100 (+)	100 (−)	100 (−)	100 (−)	100 (−)
Mayette	0.1 (+ + + +)	10 (+ +)	0.1 (+ + +)	100 (−)	100 (−)	100 (−)	100 (−)	100 (−)
Mellanaise	0.1 (+ + + +)	0.1 (+ + + +)	0.1 (+ + + +)	100 (−)	100 (−)	100 (−)	100 (−)	100 (−)
Parisienne	0.1 (+ +)	0.1 (+ + + +)	0.1 (+ + + +)	100 (−)	100 (−)	100 (−)	100 (−)	100 (−)

No antimicrobial activity (−), inhibition zone < 1 mm. Slight antimicrobial activity (+), inhibition zone 2–3 mm. Moderate antimicrobial activity (+ +), inhibition zone 4–5 mm. High antimicrobial activity (+ + +), inhibition zone 6–9 mm. Strong antimicrobial activity (+ + + +), inhibition zone >9 mm. Standard deviation ± 0.5 mm.

Gram positive bacteria were the most sensitive being inhibited by all the extracts. Concerning Gram negative bacteria, only the extracts of *cv.* Franquette and *cv*. Marbot were able to inhibit the growth of *P. aeruginosa* at the highest extract concentration tested (100 mg/mL); fungi were resistant. The pure active compounds ampiciline and cycloheximide presented lower MICs than the walnut husk extracts.

The extracts presented similar antimicrobial capacity, inhibiting Gram + bacteria and in the order *S. aureus* > *B. cereus* > *B. subtilis*. *S. aureus* was the most susceptible microorganism, presenting MICs of 0.1 mg/mL. These results are very important considering that *S. aureus* can produce several types of enterotoxins that cause gastroenteritis, which is a major food-borne disease in most countries (Halpin-Dohnalek and Marth, 1989). Natural products may be a particularly rich source of anti-infective agents. For example, flavonoids showed antimicrobial activity, and quercetin and other related compounds acts essentially by enzyme inhibition of DNA gyrase (Cushnie and Lamb, 2005).

The obtained results are similar, and in some cases better, than previous results obtained with different aqueous extracts of walnut leaves (Pereira et al., 2007b). The MICs were very similar but two of the tested extracts in this work showed inhibition of *P. aeroginosa*, which was not observed with leaves extracts. On the other hand, extracts of walnut fruits (Pereira et al., in press) were much less effectives.

In conclusion, the results obtained in our work showed that walnut green husks can be used as an easily accessible source of natural bioactive compounds. We demonstrate for the first time, as far we known, that walnut green husks aqueous extracts presented a strong antioxidant activity and inhibited the growth of different pathogenic bacteria (Gram +) that can causes health problems. Further studies will be developed to characterize walnut green husks extracts and to identify the molecules responsible for this bioactivity. On other hand, the potential showed by walnut green husks extracts can lead to the valorization of a by-product, that nowadays has a scarce use.

Conflict of interest statement

The authors declare that there are no conflicts of interest.

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